Appendix

SUPPLEMENTARY MATERIALS AND METHODS

Mice

Wild type (*Hdh*$^{Q7/Q7}$), mutant homozygote (*Hdh*$^{Q111/Q111}$) or heterozygote (*Hdh*$^{Q7/Q111}$) mice of 6-9 months of age were used for HTT intramolecular interaction experiments (Wheeler et al, 1999). 20 months old mice were used for ER stress analyses and electron microscopy studies. Animals were maintained with access to food and water *ad libitum* in a colony room kept at a constant temperature (19°C-22°C) and humidity (40%-50%) on a 12:12 h light:dark cycle. Studies were performed in an authorised establishment (Institut Curie, Orsay facility license #C91471108, Feb 2011) under the supervision of authorised investigators (permission #91-448 to S. Humbert). This study was evaluated and approved by the Comité d'Ethique en matière d'Expérimentation Animale Paris Centre et Sud (National registration number: #59) presided by Pascal Bigey.

Drosophila

ELaV-Gal4 flies are gift from J.R Martin (Gif-sur-Yvette, France). UAS-TEV and hs-TEV flies are gifts from R. Schuh (Max-Planck-Institut für biophysikalische Chemie, Göttingen, Germany). The following pENTRY constructs: pARIS-HTT-N[His, mCherry]-Q100- or -Q23-167/586TEV (described later) were recombined in pTW (pUAST Gateway vector, Invitrogen) to generate transgenic *Drosophila* expressing the FL-HTT-TEV by regular P-element insertion transgenesis in a *w*^{1118} host line (Bestgene, USA). The following flies were obtained: *UAS-FL-HTT167/586TEV-Q100 and -Q23.* We used the pARIS-HTT-N[His, Cherry]-1722-3144-C[HA-TC] construct subcloned into pTW to generate transgenic *Drosophila* expressing the C-HTT1722-3144 fragment (*Elav-Gal4; UAS:C-HTT1722-3144*). pUASt-HTT548aa-Q0 and pUASt-HTT548aa-Q128 constructs (a gift of J Troy Littleton, Cambridge, USA (Lee et al, 2004)) were used to generate *UAS-N548-HTTQ0* and *UAS-N548-HTTQ128* transgenic flies.

The FL-HTT-Q23, FL-HTT-Q200, FL-HTT-167TEV-Q200, FL-HTT-586TEV-Q200 and FL-HTT-167/586TEVQ200 were derived from the pARIS-FL-HTT. An optimal *Drosophila* Kozak sequence was added before the ATG and the 23 N-terminal glutamines were replaced by a Kazantsev sequence coding for 200 glutamines. These constructs where then subcloned into a P[acman] vector (PMID: 17138868) carrying 5'UAS sequences. Transgenic strains were generated following standard procedures using the phiC31 integrase into the attP2 site in Chr. 3L (PMID: 15126397). All flies were reared on standard cornmeal food at 25°C. To obtain the double transgenic lines- UAS-TEV/UAS-TEV; HTT-TEV/HTT-TEV or hs-TEV/hs-TEV; HTT-TEV/HTT-TEV respectively on chromosome 2 and 3- we used intermediate strains containing the following balancers: curly and sparatus on chromosome 2, Drop and TM3 on chromosome 3. "hs" stands for heat shock. For all crosses, *ELaV-Gal4* virgin females were crossed with males of each of the UAS-HTT strains.

Drosophila **lifespan assays**

For Fig 5C: Two replicates of 30 female virgins each were aged for the duration of the experiment. Animals were raised and aged at 28.5°C.For Fig 5D & 5E: one to three replicates of total offspring were aged for the duration of the experiment. Flies were raised and aged at 29°C. Vials were changed three times a week and the number of dead flies was scored each second day. Two to three independent assays were performed for each strain. Animals were transferred daily into new food daily. Vials were counted every day for the duration of the experiment to score dead animals.

Preparation of ***Drosophila*** **protein lysates and immunoblottings**

To validate the transgene expression, generated flies were snap frozen with liquid nitrogen at 4 days of age. Total heads were mechanically lysed in 25 mM Tris-Hcl pH 7.4, 5 mM EDTA, 250 mM NaCl, 0.1% Triton X-100. The lysate was centrifuged for 10 min at 10000 g and supernatants were subjected to SDS-PAGE and immunoblotting. For immunoprecipitation experiments, 50 heads of adult flies were lysed, and volume was adjusted to 1 ml. Samples

were incubated with G-sepharose beads (SIGMA P3296) coupled to anti-mCherry antibody for 4 h at 4°C. Beads were then washed, re-suspended in 2X loading Buffer, heated and analysed by SDS-PAGE.

Drosophila motor performance tests

For Figure 5B: Fifteen age-matched virgin females were placed in a vial and tapped down. The number of flies that climbed up 7cm in 20s was recorded. We repeated this ten consecutive times and the average of the ten observations was plotted for each day as shown in the chart. Two replicates were tested in parallel for each genotype. The UAS-FL-HTT constructs and UAS-TEV were expressed in the nervous system using elav-Gal4. Animals were raised and aged at 28.5 °C. For Figure EV5D: During a month and three times a week, female flies (>4 days of ages) were put in a closed empty vial of 8cm high and gently tapped to the bottom of the vial and the number of flies failing to climb to the top within 30 seconds was scored. Ten trials were performed at each time point. Around 40 flies were subjected to the test for each genotype. The UAS-HTT constructs were expressed in the nervous system using elav-Gal4.

siRNA

The following siRNA were used against HTT: ACA AUA AGG UCA CCU UAG A or GCA GGU UUU AGA UUU GCU G (Eurogentec) and Beclin1: (SASI_Mm01_00048143 and 00048144; Sigma-Aldrich) at a final concentration of 100μM. The control siRNA used is a universal negative siRNA (eurogentec OR-0030-neg05) that does not interfere with any known sequence of the murine genome.

Dynamin 1 constructs

Dynamin 1 WT and K44A constructs correspond to human wild-type (WT) dynamin 1 and GTP inactive mutant K44A respectively. Both constructs were fused to eGFP (peGFP-DNM1, Addgene). Constructs encoding dynamin 1 targeted to endoplasmic reticulum membranes,

termed ER-DNM1-WT and ER-DNM1-K44A correspond to human dynamin1 WT (DNM1-WT) or inactive mutant K44A (DNM1-K44A) fused to the endoplasmic reticulum transmembrane domain of human atlastin 1 at the C-terminal part. The following cDNA that contains a human kozak sequence, a HA tag, AscI, NotI and EcoRI restriction sites and the C-terminal transmembrane domain of atlastin-1 (TM, aas 446 to 558). Kozak sequence, HA and TM are hereafter underlined: A CCG GTC ACC -ATG GCC TAC CCC TAT GAT GTG CCA GAC TAC GCC CGG CGC GCC GCG GCC GCA CGA ATT CCA GCC ACA CTG TTT GTA GTC ATC TTT ATC ACA TAT GTG ATT GCT GGT GTG ACT GGA TTC ATT GGT TTG GAC ATC ATA GCT AGC CTA TGC AAT ATG ATA ATG GGA CTG ACC CTT ATC ACC CTG TGC ACT TGG GCA TAT ATC CGG TAC TCT GGA GAA TAC CGA GAG CTG GGA GCT GTA ATA GAC CAG GTG GCT GCA GCT CTG TGG GAC CAG GGA AGT ACA AAT GAG GCT TTG TAC AAG CTT TAC AGT GCA GCA GCA ACC CAC AGA CAT CTG TAT CAT CAA GCT TTC CCT ACA CCA AAG TCG GAA TCT ACT GAA CAA TCA GAA AAG AAA AAA ATG TAA GGC CGG CCA TAC GTA. This sequence was cloned in a pUC19 vector surrounded by AgeI and FseI/SnaBI sites. The human DNM1 WT or K44A cDNA subcloned in a pENTRY vector were digested by AscI and NotI and dynamin 1 cDNA was subsequently inserted into the pUC19-HA-TM-COOH, generating a pUC19-HA-DNM1-TM construct. This construct was then digested and the AscI-EcoRI fragment was subcloned in a pENTRY Gateway vector. A recombination into the pCDNA3.2DEST vector was performed to obtain vectors expressing ER-DNM1-WT and ER-DNM1-K44A.

FL-HTT-TEV constructs

The FL-HTT-TEV constructs encode the human HTT protein containing a TEV recognition cleavage site (TEVrcs) at specific cleavage positions. These HTT-TEV constructs derive from the pARIS synthetic gene (pARIS-FL-HTT) previously generated and whose sequence is published. pARIS-HTT contains unique restriction sites every kb to facilitate cloning. This construct is insensitive to several siRNAs from various species and encodes a fully functional HTT protein (Pardo et al, 2010). Amino-acids corresponding to the endogenous cleavage

sites of caspase-6 (D_{586}), caspase-3 ($D_{510}SVDL_{514}$), cathepsinZ/bleomycin hydrolase (R167) were replaced by the recognition cleavage site (ENLYFQS) of the TEV protease. Mutations were done by mutagenesis on HTT fragments cloned in pUC19 vector(Pardo et al, 2010). Replacement of R167 by the TEVrcs, was done by directed mutagenesis on the pUC19-F2 using the following primers: GAC AGC AAC CTT CCA GAA AAC CTG TAC TTC CCA GTC CCT ACA GCT CGA ACT G (sens) and CAG TTC GAG CTG TAG GGA CTG GAA GTA CAG GTT TTC TGG AAG GTT GCT GTC (antisens). Replacement of D586 by the TEVrcs was done on the pUC-F3 using the following primers: TCT GAA ATT GTG TTA GAA AAC CTG TAC TTC CAG TCC GGT ACC GAC AAC CAG (sens); CTG GTT GTC GGT ACC GGA CTG GAA GTA CAG GTT TTC TAA CAC AAT TTC AGA (antisens).

The pUC19-F2 constructs mutated for cathepsinZ/bleomycin hydrolase site (167) and the pUC19-F3 constructs mutated for caspase-3 (513) or caspase-6 (586) cleavage sites were digested respectively by SacI/SacII and SacII/KpnI and cloned in pARIS-HTT-N[His-mCherry]-Q88- or –Q23-C[□stop]. Constructs were digested by SmaI/SnaBI and religated to remove the SmaI/SnaBI fragment containing HA tag, TC tag and the stop codon. FL-HTT constructs containing two TEVrcs (FL-HTT-167/586TEV, FL-HTT-167/513TEV) were subsequently obtained by a SacI/SacII digestion of pUC19-F2-167TEV vector and by a SacII/KpnI digestion of pUC19-F3-513TEV or pUC19-F3-586TEV. Digested fragments were subcloned in pARIS-HTT-N[His-mCherry]-Q23-C[□stop] or pARIS-HTT-N[His-mCherry]-Q88-C[□stop]. Each of the engineered HTT-TEV sequences, inserted into a pENTRY vector, was transferred into the pCDNA-DEST47 Gateway vector using the LR-clonase-II (Invitrogen). All the constructs were verified by sequencing prior use and are referred as follows with the CAG numbers indicated:

pARIS-HTT$^{pcDNADEST3.2}$ N[His-mCherry]Q100-C[HA-TC]: FL-HTT-Q100

pARIS-HTT^{pcDNADEST47} N[His-mCherry]Q100-C[GFP]: FL-HTT-Q23

pARIS-HTT^{pcDNADEST47} N[His-mCherry]Q100-C[GFP]: FL-HTT-Q100

pARIS-HTT^{pcDNADEST47} N[His-mCherry]Q23-167TEV-C[GFP]: FL-HTT167TEV-Q23

pARIS-HTT^{pcDNADEST47} N[His-mCherry]Q88-167TEV-C[GFP]: FL-HTT167TEV-Q100

pARIS-HTT^{pcDNADEST47} N[His-mCherry]Q88-513TEV-C[GFP]: FL-HTT513TEV-Q100

pARIS-HTT^{pcDNADEST47} N[His-mCherry]Q88-586TEV-C[GFP]: FL-HTT586TEV-Q100

pARIS-HTT ^{pcDNADEST47} N[His-mCherry]Q88-167/513TEV-C[GFP]: FL-HTT167/513TEV-Q100

pARIS-HTT^{pcDNADEST47} N[His-mCherry]Q23-167/586TEV-C[GFP]: FL-HTT167/586TEV-Q23

pARIS-HTT^{pcDNADEST47} N[His-mCherry]Q88-167/586TEV-C[GFP]: FL-HTT167/586TEV-Q100

N and C-terminal HTT constructs

HTT N-terminal constructs encoding the first 586 aas of the protein, are referred as N586-HTTQ23 or Q100 and were generated from pARIS-HTT-N[His, Cherry]Q23-1-586-C[FKBP] corresponding to the sequence of FKBP fused to the first 586 aas of HTT protein. A stop codon was inserted by PCR at position 586. Following primers were used: GAA ATT GTG TTA GAC TAA ACC GGA GTG CAG GTG GAA (sens) and TTC CAC CTG CAC TCC GGT TTA GTC TAA CAC AAT TTC (antisens). The resulting construct was termed pARIS-HTT-N[His-mCherry]Q23-586Stop. To obtain pARIS-HTT-N[His-mCherry]Q100-586Stop, we deleted the NotI/SacI fragment and replaced by the NotI/SacI fragment from pARIS-FLHTT-Q100. N-terminal HTT construct encoding the first 167 aas of HTT and referred as N167-HTTQ100 was generated by the insertion of a stop codon at the position 167 in the construct pARIS-HTT-N[His-mCherry]Q100-586Stop to generate pARIS-HTT-N[His-mCherry]Q100-167Stop. The following primers were used for the PCR: AAC CTT CCA AGG TAA TAG CCC GGG CTG TAT AAG GA (sens), TCC TTA TAC AGC CCG GGC TAT TAC CTT GGA AGG TT (antisens). The N586-HTT167TEVQ100 and N586-HTT167TEVQ23 constructs (pARIS-HTT-N[His-mCherry]Q100-167TEV-586Stop or –Q23) were generated by subcloning the SacI/SacII fragment containing the mutation 167TEV in pUC19-F2 into pARIS-HTT-N[His-mCherry]Q100-586Stop or -Q23.

The C-HTT587-3144 construct express the 587 to 3144 aas fragment of HTT protein. To generate such construct, we first inserted a KpnI site at position 586 and simultaneously deleted the KpnI site at aa 1051 from pARIS-FL-HTT-Q23 construct by performing a step of

three PCR using the indicated primers: 1) GGT TGG CAC TGT GGA GTG CCG CCA CTG AGT (sens); 2) GGC ACT CAG TGG CGG CAC TCC ACA GTG CCA ACC (antisens); 3) CTG ATC CAG TAG TGG CCG TGG CAA GAG ATC (sens) and 4) AAC ACA AAC AGT TGC CAT CAT TGG TTC TCT (antisens). The first round of PCR was done using the set of primers 2 and 3, the second round using the set of primers 1 and 4. The third PCR was performed using the fragment produced by the two previous PCR with the set of primers 3 and 4. We thus obtained an intermediate product surrounded by NsiI-XbaI sites. This fragment was cloned in a pCR4-TOPO vector (Invitrogen), digested by AatII-XbaI in order to deplete KpnI site at position 586 and finally cloned in pARIS-FL-HTT vector.

In order to insert a KpnI site at position 586, we similarly performed mutagenesis steps in pUC19-F3 vector using the following primers: GAA ATT GTG TTA GAC GGT ACC GAC AAC CAG TAT TTG G (sens) and CCA AAT ACT GGT TGT CGG TAC CGT CTA ACA CAA TTT C (antisens). The generated fragment containing a KpnI site at position 586 was then cloned into the same pARIS FL-HTT using SacII-AatII restriction enzymes. Finally, the N-terminal part of HTT (aas 1 to 586) was deleted by NotI/KpnI digestion, blunting reaction and ligation to obtain pARIS-HTT-N[His-mCherry]-587-3144-C[HA-TC] construct.

The C-HTT1722-3144 construct (pARIS-HTT-N[His-mCherry]-1722-3144-C[HA-TC]) express the 1722 to 3144 aas fragment of HTT protein. This construct termed pARIS-HTT-N[His-mCherry]-1722-3144-C[HA-TC] was generated from pARIS-FL-HTT-Q23 construct by NotI/ScaI digestion, blunting reaction and ligation. Each of the engineered N and C-terminal pENTRY-pARIS-HTT constructs were recombined into the pCDNADEST3.2 Gateway vector (Invitrogen). The pEGFPC3-C-HTT587-3144 construct was generated from a plasmid encoding a C-terminal fragment of human HTT (a gift from Nicole Déglon, Lausanne University Hospital, Lausanne, Switzerland). The KpnI/SacII fragment was excised and ligated into a modified version of pEGFP-C1. We introduced a spacer consisting of a 1.2 kb SacII fragment from a YFP-HTT480-Q17 plasmid into pEGFP-C1. This modified pEGFP-C1 was used for the insertion of the KpnI/SacII fragment coding for the HTT C-terminal HTT fragment.

Cell lines and transfection procedures

HEK293T and HeLa cells were grown at 37°C, 5% CO_2, in a standard media containing DMEM (Dulbeco's modified Eagle's medium, Gibco) supplement with 2 mM of L-glutamine, 1% sodium pyruvate and 10% of inactivated bovine calf serum. HEK293T cells were transfected by calcium phosphate method. HeLa cells were transfected using Lipofectamine 2000 reagent according to the guidelines. In both cases, the DNA complexes were added to cells and left for 20 h to 24 h.

ST*Hdh*$^{Q7/Q7}$ cells are immortalised striatal neurons from *Hdh*$^{Q7/Q7}$ mice (Trettel et al, 2000). Striatal cells were grown at 33°C, 5% CO_2, in DMEM supplemented with 1% of non-essential amino-acids, 2mM L-glutamine and geneticin (400 µg/ml) and transfected using Lipofectamine 2000 according to the guidelines or by electroporation using the cell line nucleofactor kit L (LONZA) according to the manufacturer's instructions.

Reagents

Salubrinal (324895), Z-DEVD-FMK (264155) and Z-VAD-FMK (6276105) are from Calbiochem. Tunicamycin (T7765), ADP (A4386), proteases (P8340) and phosphatases (P5726) inhibitors cocktails are from Sigma-Aldrich. Disuccinimidyl suberate (DSS, 68528-80-3) is from Thermo scientific. Rapamycin (1292) is from TOCRIS.

HTT proteolysis

For *in vitro* cleavage of HTT by recombinant TEV protease, HEK293T cells were transfected with the different HTT constructs encoding FL-HTT containing TEVrcs at specific positions. 24h later, cell were lysed in 20 mM Tris-HCl at pH 8.0, 120 mM NaCl, 1 mM EDTA, 0.5% NP40 without protease inhibitors and then centrifuged for 10 min at 13,000 rpm. 50 µg of total proteins were incubated 2 h at 34°C with 10 µg of the recombinant TEV (gift from C. Janke laboratory), in 50 m Tris-HCl pH 7.5, 1 mM EDTA, 150 mM NaCl. Proteolytic reactions

were stopped by addition of proteases inhibitors and Laemmli loading buffer (5X). Samples were subjected to SDS-PAGE and immunoblotting.

For HTT cleavage within cells, HEK293T or ST*Hdh*$^{Q7/Q7}$ cells were co-transfected with the FL-HTT and the SNIPer-TEV plasmids or a pcDNA empty vector for control condition. After 24h, cells were treated with rapamycin (10 or 20 nM) for 24h. Cell lysates were centrifuged as before, and analysed by immunoblotting using mini-protean TGX precasted gels of 4-15% (BIORAD, 4561086).

Post-mortem human samples were obtained from the Harvard Brain Tissue Resource Center (HBTRC; Belmont, MA): two controls (samples 1-2, age: 53 and 54 years old respectively; post-mortem delay: 22.2 ± 2 h), one HD grade 4 (sample 3), and one HD grade 3 (sample 4) patients (45 and 82 years old respectively; post-mortem delay: 22.3 ± 0.3 h). Samples correspond, respectively, to brain numbers B4744, B4751, B4740 and B4797 as numbered by HBTRC. Samples were homogenised in 20mM Tris pH8, 120mM NaCl, 1mM EDTA and 0.5% NP-40 lysis buffer and cleared by centrifugation at 6 000 g (15 min) (Godin et al, 2010).

Immunoprecipitation

To study HTT fragment interaction in vivo, *Hdh*$^{Q7/Q111}$ mice were decapitated and brain was rapidly removed and in 20 mM Tris-HCl pH8; 120 mM NaCl; 1 mM EDTA ; 0.5% NP40, without protease inhibitor. After passing through a 25-gauge needle, the homogenate was centrifuged twice for 10 min at 13 000 rpm. Supernatant was incubated with the active recombinant human caspase 6 (Clinisciences, 1086-25) for 1h30 at 37°C using 1 mg of total proteins for 20 units of enzyme. The following immunoprecipitation procedure was used: first 40 µl of ½ slurry protein G-Sepharose beads (Sigma-Aldrich P3296) were coated with 1 µg of the indicated antibody, by incubating 2 h at 4°C in lysis buffer (20 mM Tris-HCl pH 8.0; 120 mM NaCl; 1 mM EDTA; 0.5% NP40, protease and phosphatase inhibitors). Then, antibody-coated beads were washed in lysis buffer and incubated with 1 mg of cell lysate for 2 h at 4°C. Beads were finally washed four times and immunoprecipitated proteins were resolved by SDS-PAGE and analysed by immunoblotting. Similar immunoprecipitation procedures

were used to study interaction in cells. HTT cleavage by the SNIPer-TEV was induced with 20 nM rapamycin treatment during 24 h.

HTT interaction to dynamin 1: ST*Hdh*$^{Q7/Q7}$or HEK293T cells were transfected with either of the following constructs: N-terminal HTT, C-terminal HTT, FL-HTT containing the TEVrcs, SNIPer-TEV or pcDNA, dynamin1 WT or ER-DNM-WT. In experiments requiring HTT cleavage, cells were treated with 20 nM of rapamycin during 24 h. After treatment, cells were washed twice with cold PBS on dishes. Cells were lysed in 20 mM Hepes pH 7.4, 100 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.5% Triton X-100, containing proteases and phosphatases inhibitors. Collected cells were centrifuged for 15 min at 11,300 g. Supernatant was pre-cleared with 50 µl of ½ slurry G-Sepharose beads (Sigma-Aldrich P3296) during 1 h at 4°C. 1 µg of anti-mCherry antibody and 5 mM ADP (sigma A4386) were added to the pre-cleared lysate and left under rotation overnight. G-beads were then added to samples for 3 h and 30 min at 4°C (beads were primarily blocked with 5% BSA to reduce unspecific binding). Post-mortem human samples were incubated with 1 µg of anti-C-ter HTT (D7F7) and 50 µl of ½ slurry G-Sepharose beads (Sigma-Aldrich P3296) during 1 h at 4°C. After washing, beads were resuspended with 2X loading buffer then analysed on a SDS-PAGE followed by immunoblotting.

Yeast two-hybrid analysis

Yeast two-hybrid screening was performed by Hybrigenics Services, S.A.S., Paris, France (http://www.hybrigenics-services.com). The coding sequence for human HTT aas 1230 to 3144 (GenBank accession number gi: 90903230) was PCR-amplified and cloned into pB66 as a C-terminal fusion to Gal4 DNA-binding domain (N-Gal4-HTT-C). The constructs were checked by sequencing and used as a bait to screen a random-primed Human Adult Brain cDNA library constructed into pP6. pB66 and pP6 derive from the original pBTM116 (Vojtek & Hollenberg, 1995), pAS2ΔΔ and pGADGH plasmids (Fromont-Racine et al, 1997), respectively. For the Gal4 construct, 59.7 million clones (5 fold the complexity of the library) were screened using a mating approach with HGX13 (Y187 ade2-101::loxP-kanMX-loxP,

mat) and CG1945 (mata) yeast strains as previously described (Fromont-Racine et al, 1997). A total of 116 His+ colonies were selected on a medium lacking tryptophan, leucine and histidine. The prey fragments of the positive clones were amplified by PCR and sequenced at their 5' and 3' junctions. The resulting sequences were used to identify the corresponding interacting proteins in the GenBank database (NCBI) using a fully automated procedure. A confidence score (PBS, for Predicted Biological Score) was attributed to each interaction as previously described (Formstecher et al, 2005).

For further description of the confidence score: The PBS relies on two different levels of analysis. Firstly, a local score takes into account the redundancy and independency of prey fragments, as well as the distribution of reading frames and stop codons in overlapping fragments. Secondly, a global score takes into account the interactions found in all the screens performed at Hybrigenics using the same library. This global score represents the probability of an interaction being nonspecific. For practical use, the scores were divided into four categories, from A (highest confidence) to D (lowest confidence). A fifth category (E) specifically flags interactions involving highly connected prey domains previously found several times in screens performed on libraries derived from the same organism. Finally, several of these highly connected domains have been confirmed as false-positives of the technique and are now tagged as F. The PBS scores have been shown to positively correlate with the biological significance of interactions (Rain et al, 2001; Wojcik et al, 2002).

Endoplasmic reticulum (ER) isolation

HEK293T cells were co-transfected with HTT constructs along with WT dynamin 1. The protocol for ER isolation was performed as described (Bozidis et al, 2007). Briefly, cells were first washed with PBS, scraped and collected by centrifugation. Cells were subjected to breakage in an osmotic solution and then sonicated. Non-lysed cells were removed by centrifugation (1,400 g for 10 min at 4°C). To discard undesired organelles, the supernatant was centrifuged at 15,000g for 20 min at 4°C. Cleared lysate was added onto the top of the ER gradient which was then ultra-centrifuged during 70 min at 152,000 g (41Ti swing rotor)

at 4°C. Finally the ER was pelleted by ultracentrifugation at 54,000 rpm (TLA100.3 rotor) during 45 min.

GTP-binding assay

ER membranes were isolated from HEK293T cells transfected with HTT constructs and dynamin 1 as described above. ER membranes were lysed in reaction buffer containing 20 mM Hepes pH 7.4, 100 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 0.5 % Triton X-100, proteases and phosphatases inhibitors cocktail and then incubated on ice for 20 min. After centrifugation at 11,300g for 15 min at 4°C, supernatant was incubated with GTP-agarose beads (Innova Biosciences 505-0001) or control resin (Innova Biosciences 520-0002G) overnight at 4°C. After washing, the bounds proteins on beads were eluted in the reaction buffer containing 10 mM GTP, as a competing ligand, for 2h at 4°C. Eluates were subjected to SDS-PAGE analysis.

Cross-linking of dynamin 1

Total cell lysates were pre-incubated for 15 min at 37°C and then incubated for 1 min at room temperature (RT) after adding 1 mM DSS. The reaction was stopped by adding 100 mM Tris-HCl pH 8.0 for 15 min at RT. Loading buffer was added and samples were subjected to SDS-PAGE analysis and immunoblotted for dynamin1.

Transferrin assay

ST*Hdh*$^{Q7/Q7}$ cells were transfected with empty mCherry, mCherry-C-HTT587-3144, or GFP-tagged dynamin 1 K44A. 24 h later, cells were starved by incubation in DMEM media for 30 min at 33°C. Cells were harvested and washed with PBS. The experiment was entirely conducted in a cold room to correctly monitor endocytosis. Surface cell labelling was achieved by incubating the cells in 2 ml of DMEM + 1 % BSA + 20 mM Hepes + 5 µg/ml A647 labelled for 1 h and mixing gently from time to time to ensure efficient binding of transferrin to its receptors. Cells were washed with DMEM, 1 % BSA, 20 mM Hepes and then

pelleted. To start endocytosis measurement, cells were put in a water bath at 37°C and aliquots were taken at different time points and endocytosis of transferrin stopped by cold PBS. When all time points were collected, cells were pelleted and submitted to an acid treatment for 5 min (50 mM Glycine, 100 mM NaCl, pH 3.0 (stripping)) to detach the remaining transferrin at the cell surface. Finally cells were pelleted and the intensity of A647 transferrin was quantified on a FACSAria^{TM}III (BD Biosciences). A mean intensity of the A647 fluorophore was calculated for 10000 cells co-transfected with HTT-mCherry or dynamin 1-GFP. The background value was settled as the following: cells were labelled with A647-transferrin and submitted to the stripping buffer with no endocytosis step in between. The mean of intensity was considered the background value and was subtracted from all other time points.

Golgi reassembly experiment

HeLa sh-HTT cell lines were transfected with FL-HTT or FL-HTT167/586TEV mCherry constructs and treated as previously described (Pardo et al, 2010) with 4 µM nocodazole for 30 min at 4°C and 90 min at 37°C to allow a complete depolymerisation of microtubules. Cells were washed twice with DMEM and incubated for 2 h at 37°C prior to methanol fixation (2 min at -20°C) and GM130 (BD transduction laboratories) immunolabelling. HeLa cells expressing mCherry were acquired and images were analysed with ImageJ software using 3D object counter.

Toxicity and vacuolation analyses

Cell death was assessed by the condensation of the nucleus stained with DAPI under an inverted fluorescent microscope. For vacuolation assessment, cells displaying a large number of vacuoles within their cytoplasm were considered vacuolated. Cells were treated with Salubrinal (21 µM) Z-DEVD-FMK (10 µM) and Z-VAD-FMK (50 µM) for 48 h. TUNEL assay was done using the in situ cell death detection kit (Roche, 12 156 792 910) and following the guidelines instructions. To assess toxicity after HTT cleavage, we used the

following procedure: ST*Hdh*$^{Q7/Q7}$ cells were electroporated with the indicated HTT-TEV constructs. 20 h later, cells were treated with 10 nM of rapamycin and tracked with live video microscopy (DMI6000B Leica), using the mCherry tag. Snap shots of each cell were taken every 4 min during 24h. The time of death was registered and survival curves were calculated as a percentage. Dying cells were recognised by shrinkage, excessive blebbing and detachment from the plate.

dSTORM imaging

Optical Setup: All dSTORM images were acquired on a Nikon Eclipse Ti inverted microscope equipped with Perfect Focus System and configured for these studies in oblique incidence excitation. Samples were excited with 488 nm and 561 nm solid-state lasers (Genesis MX-STM 500 mW, Coherent) depending on the fluorophore used. The following full-multiband laser filter sets, optimised for 488 or 561 nm laser sources (GFP-1828A-000 and TxRed-4040C-000, Semrock) were respectively used to excite Alexa Fluor 488 or Alexa Fluor 568 (stained with dynamin 1 or calnexin) and collect their fluorescence thanks to a Nikon APO TIRF 60x, 1.49 NA oil immersion objective lens. All images were recorded onto a 256x256 pixel region of an EMCCD camera (iXon 897, Andor) positioned on the focal plane of an optical telescope for optimal PSF sampling (magnification by 2.7, optical pixel size of ~100 nm). The 488 nm and 561 nm laser's powers were used at 50 mW (powers measured in the BFP). For each dSTORM image, the integration time and the EMCCD Gain of all raw images (~15000 recorded) were respectively set to 100 ms and 300.

Imaging buffer: dSTORM measurements were performed in an imaging buffer allowing the fluorophores blinking and reducing their photobleaching (van de Linde et al, 2011; Vaughan et al, 2012). This buffer contained 50-100 mM of ß-mercaptoethylamine (MEA; Sigma-Aldrich) and an oxygen scavenger system (0.5 mg.ml-1 glucose oxidase (Sigma-Aldrich), 40 µg.ml-1 catalase (Sigma-Aldrich) and 10% (w/v) glucose) dissolved in a buffer composed of 100 mM Tris-HCl (Sigma-Aldrich), 1 mM ascorbic acid (Sigma-Aldrich) and 1 mM methyl viologen (Sigma-Aldrich). The pH of the final solution was adjusted to 7.5.

Super-localisation software: Real time super-localisation was performed thanks to our home-made software written in Python, using PyQt, Numpy and Scipy libraries, which is based on image wavelet-segmentation and centroid determination (Izeddin et al, 2012). This algorithm allows us to obtain STORM images with a localisation precision of 20 nm Moreover to improve the viewing; each single-molecule detection is added to the final STORM image as a Gaussian of 15 nm width.

Immunofluorescence

ST*Hdh*$^{Q7/Q7}$ cells were electroporated with HTT constructs and plated on coverslips for 16 h, 24 h or 48 h. Antibodies used are: anti-calnexin (Sigma C4731, 1/200); anti-GFP (Millipore AB16901); anti-dynamin1 antibodies (Santa Cruz sc-12724, 1/50); anti-LAMP-2 (Santa Cruz sc-18822, 1/250); anti-HA (Roche 11867423001, 1/100); anti-mCherry (Institut Curie, 1/500); anti-ATF6 (abcam ab11909, 1/100); anti-EEA1 (Millipore 07-1820, 1/50); anti-GM130 (BD transduction laboratory, 610822, 1/500); CTR 433 (gift from P.Chavrier, Institut Curie, Paris); and anti-TGN38 (BD biosciences 610899, 1/100). All secondary antibodies used are coupled to Alexa Fluor 488, 555, 586 or 647 and purchased from molecular probes (Invitrogen).

Cells were fixed with 4% paraformaldehyde warmed up at 37°C for 20 min at RT. For calnexin, GFP-KDEL and dynamin 1 staining, ST*Hdh*$^{Q7/Q7}$ cells were fixed with cold methanol for 2 min at -20°C, washed with PBS and then treated with 2% paraformaldehyde. After the blocking step (PBS, 0.1 % Triton X-100 and 3% Bovine calf serum), first antibodies were incubated with the blocking solution overnight at 4°C. Coverslips were washed and secondary antibodies (1/200) were incubated for 2 h at RT. Cells were counterstained with DAPI, post-fixed with 2% paraformaldehyde and mounted with Mowiol anti-bleaching solution. For LAMP-2 staining, blocking solution contained PBS, 0.2% saponin and 3% Bovine calf serum. For ER markers and dynamin 1 staining, cells were blocked with PBS, 0.3% Triton X-100 and 5% normal goat serum during 1 h 30 at RT.

Electron microscopy

For ultrastructural studies, cell monolayers and/or small fragments of tissues were fixed for 1 h at 4°C with 2% glutaraldehyde in 0.1 M Sörensen phosphate buffer, pH 7.3. Cells were scraped during fixation and centrifuged. The fixed pellets and/or the tissues were rinsed for 1h in ice-cold phosphate buffer, post-fixed with 2% aqueous osmium tetroxide (EMS) and dehydrated in increasing concentrations of ethanol prior to Epon embedding. Polymerisation was carried out for 48 hours at 64°C. For observation, ultrathin sections of 90 nm were done using a Reichert Ultramicrotome III, were collected on 200-mesh grids coated with Formvar and carbon, stained with uranyl acetate and lead citrate and observed with a FEI Technai Spirit transmission electron microscope at 80 Kv. Digital images were taken with a SIS MegaviewIII CCD camera.

Immunoelectron microscopy

Transfected cells, fixed either in 2% glutaraldehyde or in 4% paraformaldehyde in 0,1M Sorënsen's phosphate buffer pH7.3, were dehydrated in methanol and embedded in Lowicryl K4M (Polysciences Europe, Germany). For GFP or dynamin 1-GFP immunolocalisation, ultrathin sections were reacted with rabbit polyclonal anti-GFP (abcam290 diluted 1/200 in PBS) for 1 hour at room temperature, washed in PBS and incubated 30 min with goat anti-rabbit antibody (1/25 in PBS) coupled to 10 nm gold particles (BBInternational). After staining with 4% uranyl acetate the sections were analysed with a FEI Tecnai Spirit electron microscope. Images were taken with a SIS MegaviewIII CCD camera.

Images acquisition and analysis

Images were acquired using a Leica DM RXA microscope with a HCL PL APO CS oil 63 x NA of 1.4 objective or a HCL PL APO CS oil 100x NA of 1.4 objective coupled to a piezzo and a coolSNAP HQ camera monitored by Metamorph software. Z-stack step was settled to 200 nm; or an inverted microscope (Eclipse Ti; Nikon) with a 100X 1.4 NA oil immersion objective coupled to a spinning-disk confocal system (CSUX1;Yokogawa) fitted with an EM-

CCD camera (Evolve; Photometrics) coupled to a piezzo monitored by Metamorph software. Z-stack step was settled to 100 nm. Maximum intensity projection of the fluorescent channels was performed. Images were treated with ImageJ (Rasband, W.S., ImageJ, U. S. National Institutes of Health, Bethesda, Maryland, USA, http://imagej.nih.gov/ij/, 1997-2012).

For live-cell imaging, cells were recorded by a Leica DMI6000 B microscope with an air-40X objective coupled to a moving stage and a photometric camera eVolve (Photometrix) controlled by Metamorph software. Imaging was performed at 37°C in 5% CO2 and cells were excited using a mCherry filter using an exposure time of 500 ms. For HTT fragments co-localisation studies, quantification was achieved using the ImageJ JACoP plugin (Bolte & Cordelieres, 2006). For immuno-EM images, GFP immuno gold dots localised at the border of an ER tubule were counted and a mean of density (number of dots/area of tubule μm^2) was performed.

Immunoblotting experiments

Equal amount of proteins was subjected to SDS-PAGE on polyacrylamide gels and transfer to PVDF membranes (Millipore IPVH00010). Membranes were saturated with 5% BSA or 5% dry milk in TBS-0.1% Tween-20 for 30 min at RT. Primary antibodies were incubated for 2 h at RT or overnight at 4°C. Antibodies used are: anti-calnexin (sigma C4731, 1/2000); anti-HA (Roche 11867423001, 1/1000); anti-mCherry and anti-GFP (Institut Curie, 1/500); anti-dynamin 1 (Novus Biological, NB110-56933, 1/1000); anti-ATF6 (abcam, ab11909, 1/250); anti-polyQ mAb 1C2(Trottier et al, 1995b), 1/1000; anti-BIP/GRP78 (Santa Cruz, sc-1050, 1/1000); anti-α-tubulin (Sigma, T6199, 1/1000); anti-actin (Sigma, A5441, 1/5000); anti-eif2α (cell signalling, 5324, 1/1000); anti-eif2α pS53 (Invitrogen, 447228G, 1/1000); anti-beclin1 (Santa Cruz, sc-11427, 1/1000); anti-mitochondrial hsp70 (pierce, MA3-028, 1/400); anti-LaminC (Developmental Studies Hybridoma Bank); anti-annexin V (epitomics,2792-S, 1/1000); anti-EEA1 (Santa Cruz, sc-6415, 1/500) and anti-HTT: mAb 2B4 (Lunkes et al, 2002), 1/1000; mAb 4C8, mAb 2E8 and mAb 4E6 (Trottier et al, 1995a), 1/1000; MAB5490 (Millipore, 1/500); pAb D7F7 (CST, 1/1000).

ER stress analysis

For ER stress analysis, ST*Hdh*$^{Q7/Q7}$ cells were transfected with C-HTT587-3144 tagged with GFP and sorted 24 h later on a FACSAria^{TM}III (BD Biosciences). For positive control of ER stress induction, non-transfected ST*Hdh*$^{Q7/Q7}$ cells were treated with 2 µg/ml of tunicamycin overnight. For HTT proteolysis analysis and ER stress analysis in mouse brain, mice were decapitated and the striatum was rapidly dissected and dounce homogenised in 20 mM Tris-HCl pH 8.0; 120 mM NaCl ; 1 mM EDTA ; 0.5% NP40. After passing through a 25-gauge needle, the homogenate was centrifuged twice for 10 min at 13,000 rpm and supernatants were analysed by SDS-PAGE and immunoblotting.

Statistical analysis

Graphpad Prism version 6.02, Graphpad Instat version 3.05 and Statview 4.5 software (SAS Institute Inc.) were used for statistical analysis. The criterion for statistical significance was set up at $P<0.05$.

ANTIBODY INFORMATION

anti-HTT 2B4(Lunkes et al.,2002);
anti-HTT 4C8 (Trottier et al., 1995a),
anti-HTT 2E8 (Trottier et al., 1995a),
anti-HTT 4E6 (Trottier et al., 1995a),
anti-HTT, a.a. 115-129 (Millipore MAB5490, Monoclonal Mouse), 1DB-001-0000847198; Juenemann et al, 2011;
anti-HTT D7F7 (Cell Signaling #5656, Monoclonal Rabbit); Gusella, J.F. and Macdonald, M.E. (Trends Biochem.Sci. 2006); Borrell-Pagès, M. et al. (Cell Mol. Life Sci 2006); Luo, S. et al. (J. Cell Biol. 2005);
anti-polyQ mAb 1C2(Trottier et al., 1995b);
anti-calnexin (Sigma C4731, Polyclonal Rabbit), 1DB-001-0000826077 ;
anti-dynamin1 (Santa Cruz sc-12724, Monoclonal Mouse); 1DB-001-0000183464 ;
anti-dynamin 1, clone EP772Y (Novus Biological NB110-56933, Monoclonal Rabbit);
anti-LAMP-2, clone H4B4 (Santa Cruz sc-18822; Monoclonal Mouse); Otomo et al, (J.Biol.Chem. 2011);
anti-ATF6 (abcam ab11909; Monoclonal Mouse); 1DB-001-0000101143 ;
anti-EEA1 (Millipore 07-1820; Polyclonal Rabbit); 1DB-001-0000848475 ;
anti-GM130 (BD transduction laboratory 610822; Monoclonal Mouse); 1DB-001-0000570970 ;
anti-CTR 433 (gift from P.Chavrier, Institut Curie, Paris);
anti-TGN38 (BD biosciences 610899; Monoclonal Mouse); Mary S. et al (Mol.Biol.Cell 2002)
anti-BIP/GRP78, clone N-20 (Santa Cruz sc-1050; Polyclonal Goat); Yang Y.C. et al (Cell Death Dis. 2013);
anti-eif2α (cell signalling 5324; Monoclonal Rabbit); 1DB-001-0000862810 ;
anti-eif2α pS53 (Invitrogen 447228G; Polyclonal Rabbit); 1DB-001-0000891018;
anti-beclin1(Santa Cruz sc-11427; Polyclonal Rabbit); 1DB-001-0000184580 ;
anti-Hsp70 (Thermo Fisher Scientific Pierce MA3-028, Monoclonal Mouse); Xie.B. et al (J.Biol.Chem. et al, 2005);
anti-LaminC (Developmental Studies Hybridoma Bank);
anti-annexin V (epitomics 2792-S, Monoclonal Rabbit);
anti-α-tubulin (Sigma T6199, Monoclonal Mouse); 1DB-001-0000869177 ;
anti-actin, clone AC-15 (Sigma A5441, Monoclonal Mouse); North A.J. (J.Biol.Cell 1993);
anti-HA (Roche 11867423001);
anti-mCherry (Institut Curie);
anti-GFP (Millipore AB16901, Polyclonal Chicken); 1DB-001-0000851855

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